Dispatches

Ciliary Secretion: Switching the Cellular Antenna to 'Transmit'

Cilia are microtubule/membrane-based protrusions that mediate cell motility or transduce sensory information. New work in *Chlamydomonas* demonstrates that cilia can also act as secretory organelles by budding enzyme-containing vesicles from the flagellar membrane for post-mitotic hatching of daughters from the mother cell wall.

Prachee Avasthi and Wallace Marshall

Cilia are microtubule-based protrusions of the plasma membrane that were first noticed for their role in generating fluid flow, such as the flow of mucus in the airway. In recent decades, it has become clear that cilia also have important sensory roles and act as antennae, sensing the cell's environment: for example, kidney cilia can transduce calcium signals mediated by mechanosensitive channels sensing fluid flow [1]; photoreceptor cilia capture light and transduce visual signals to electrical signals via the G-protein-coupled receptor (GPCR) rhodopsin; and cilia from olfactory sensory neurons can detect and transduce odor stimuli also via specialized GPCRs. In addition, cilia can play roles in processing signals within cells; for example, developmental patterning of vertebrate limbs is regulated by ciliary transport of Hedgehog signaling components [2–4]. Given the varied functions of ciliary signaling, defects in conserved ciliary structure often result in disorders with seemingly unrelated pleiotropic phenotypes. A new finding reported in a recent issue of *Current Biology* by Wood *et al.* [5] reveals an interesting twist on the signaling roles of cilia, by showing that the motile flagella of the unicellular green alga *Chlamydomonas* can release biochemical signals into the extracellular environment via membrane budding of enzyme-containing ciliary ectosomes. If anyone still doubted the importance of the cilium in essential cellular functions, this new demonstration of the multitasking abilities of this nearly ubiquitous organelle should convince them otherwise.

Previous work showed that cilia formation and membrane trafficking shared common molecular components. For example, IFT20, a component of the intraflagellar transport (IFT) complex that transports core components from the basal bodies to tips of cilia, was shown to localize to the Golgi complex and play a role in the transport of the polycystin-2 cationic transmembrane channel to the plasma membrane for ciliary entry [6]. IFT20 also localizes to the Golgi and microtubule-organizing center in T cells, which do not have cilia on their surface. In these cells, IFT20 associates with IFT88 and IFT57 to mediate activity-dependent exocytosis of T-cell receptor/CD3 complexes to the plasma membrane at the interface between T cells and antigen-presenting cells [7]. Further evidence of the role of secretory pathways in ciliary function comes from the discovery of Bardet-Biedl syndrome (BBS) proteins. BBS is a ciliopathy containing many hallmarks of other cilia-related disorders, including polydactyly, retinal degeneration, kidney cysts, and obesity, and can result from mutations in 12 different genes [8]. Extension of the ciliary membrane and trafficking of membrane proteins to the cilium require a secretory pathway involving an octomeric coat complex formed by a subset of the BBS proteins [8,9].

Now, Wood *et al.* [5] report dramatic new evidence that cilia are themselves secretory organelles providing a conduit for material to flow out of the cell into the surrounding environment. In the new study, transmission electron microscopy identified 50–200 nm diameter vesicles/ectosomes surrounding flagella within a post-mitotic sporangium. *Chlamydomonas* cells cycling between light and dark grow in size during an extended G1 phase and then undergo several rounds of G2-less division resulting in many daughter cells that are trapped within the mother cell wall [10]. Daughter cells must hatch from the cell wall using the vegetative lytic enzyme contained within their flagella [11]. Immunogold labeling of the lytic enzyme demonstrated that it was contained within the ectosomes released from flagella. Immunofluorescent localization of the lytic enzyme further labeled flagella with higher concentrations at flagellar tips and in puncta localized between flagella and the mother cell wall where ectosomes would be. Finally, when biochemically purified ectosomes were harvested from hatching sporangia and added back to hatching-defective *Chlamydomonas* mutants, they allowed the cells to hatch, demonstrating the enzymatic activity of the flagella-derived ectosomes. This work highlights an important new mechanism by which cilia can enzymatically modify the external environment via a secretory pathway, demonstrating the capacity of cilia to send as well as receive appropriate signals with appropriate timing during the cell cycle (Figure 1A). The authors favor a model in which ectosomes are released constitutively but are populated with the appropriate enzyme based on regulated expression and transport, which is supported by the seemingly constitutive release of ectosomes in vegetative cells and regulated lytic enzyme expression.

Much of what we currently know about human cilia was first learned in green algae due to the genetic and biochemical advantages of the algal system, but the high degree of molecular and functional conservation between algal and mammalian cilia has meant that phenomena first seen in algae are subsequently also seen in human cilia. Intraflagellar transport is an obvious example of a molecular pathway first seen in *Chlamydomonas* flagella but later shown to be virtually identical in human cilia, but other

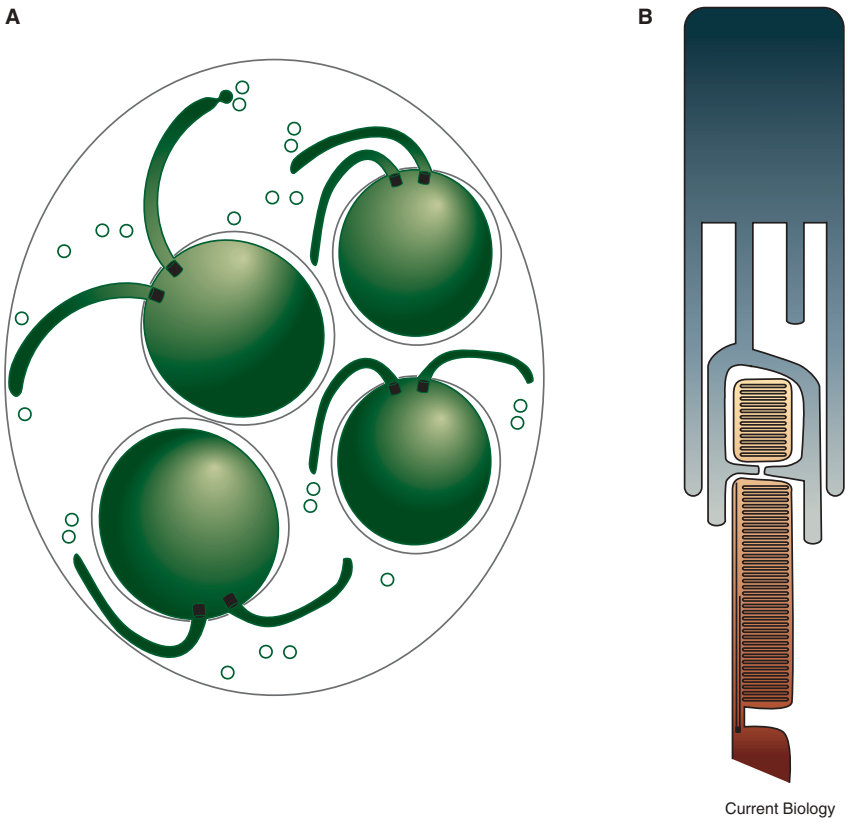

Figure 1. Membrane release from divergent cilia suggests a novel conserved secretory function for this ubiquitious organelle.

(A) *Chlamydomonas* flagella bud vesicles from distal tips that contain the vegetative lytic enzyme for hatching from the mother cell wall following mitosis. (B) Photoreceptor cilia (brown) shed aged membranous discs at distal ends, which are then engulfed and degraded with the help of the adjacent phagocytosing retinal pigment epithelium cells (blue).

examples include the conserved role of specific flagellar components, such as radial spokes or the central microtubule pair, in regulating ciliary motility. We thus have every reason to expect that the ability of cilia to function as secretory organelles is likely to hold true in mammalian cilia.

In fact, there is already a precedent for membrane release from the distal ends of cilia in the case of vertebrate retinal photoreceptors (Figure 1B). These specialized cilia contain membranous discs in their outer segments that house the transmembrane and membrane-associated proteins required for phototransduction. The discs are shed from the distal tips of cilia and phagocytosed by apical processes in the neighboring retinal pigment epithelium and shedding is timed with respect to light–dark cycles, with a burst of shedding at light onset [12]. This process is offset by the biogenesis of new protein-containing discs at the proximal end for effective turnover of the entire length of the photoreceptor outer segments about every 10 days [13]. Disc shedding is absolutely critical for the health of the cell. In a well-studied animal model for retinal degeneration, the so-called Royal College of Surgeons (RCS) rat, a mutation that prevents retinal pigment epithelial cells from phagocytosing the membranous discs causes rapid degeneration and death of the photoreceptors [14].

Export of material from the distal cilia is also required for proper sensory signaling within the photoreceptor cilium itself. During dark adaptation, a process which restores sensitivity to the bleached light-sensing photopigment, the photoisomerized chromophore is processed within photoreceptors and transported across the interphotoreceptor matrix into the retinal pigment epithelium for further processing and eventual return back to photoreceptors to be joined with the protein component of the pigment for re-use. Mutations in the enzymes of this retinoid cycle result in a wide range of retinal dystrophies, including retinitis pigmentosa and macular degeneration [15,16], highlighting the importance of the secretory functions of the distal cilia for the signaling and health of the photoreceptor cell. It has often been assumed that membrane shedding and release of molecules required for signaling from photoreceptors was a retina-specific phenomenon, but the clear demonstration of vesicle secretion and enzyme release from a more generic type of ciliary structure argues that secretory processes are a general feature of all cilia.

Another potential example of ciliary vesicle release may be urinary exosomes, vesicles that are secreted from epithelial cells lining the urinary lumen and often used as biomarkers of disease. In one instance, urinary exosomes containing several ciliary proteins were found to associate externally with cilia both from patients with autosomal recessive polycystic kidney disease and from a mouse model of the same [17]. Far fewer vesicles seem to associate with cilia in the corresponding wild-type controls. Though it has been suggested that these vesicles interact and adhere to the cilia rather than bud from them, the new evidence for ciliary ectosomes in *Chlamydomonas* should prompt a second look to ask whether these vesicles might arise by a cilia-mediated secretion system like that discovered in *Chlamydomonas*. If so, it will be critical to determine whether changes in signaling in the disease and non-disease states are reflected in varied levels of urinary exosome release from cilia.

The cilia-derived vesicles that carry proteolytic enzymes could also carry signaling molecules. *Chlamydomonas* cells signal to their mating partners through their flagella [18], providing a precedent for cilia-generated signals, but in that case the signaling is via direct contact between flagella. The ability of cilia to send signals over longer ranges suggests a more general role in developmental signaling. Ciliary secretion might also modulate internal ciliary signaling via sensitization, habituation or extrusion of unwanted or recycling material, as is the case for photoreceptor cilia. Considering all

these possible functions, ciliopathies may turn out to involve alterations in signals transmitted from cilia, not just in signals they receive.

References

1. Praetorius, H.A., and Spring, K.R. (2001). Bending the MDCK cell primary cilium increases intracellular calcium. J. Membr. Biol. *184*, 71–79.
2. Huangfu, D., and Anderson, K.V. (2005). Cilia and Hedgehog responsiveness in the mouse. Proc. Natl. Acad. Sci. USA *102*, 11325–11330.
3. Huangfu, D., Liu, A., Rakeman, A.S., Murcia, N.S., Niswander, L., and Anderson, K.V. (2003). Hedgehog signalling in the mouse requires intraflagellar transport proteins. Nature *426*, 83–87.
4. May, S.R., Ashique, A.M., Karlen, M., Wang, B., Shen, Y., Zarbalis, K., Reiter, J., Ericson, J., and Peterson, A.S. (2005). Loss of the retrograde motor for IFT disrupts localization of Smo to cilia and prevents the expression of both activator and repressor functions of Gli. Dev. Biol. *287*, 378–389.
5. Wood, C.R., Huang, K., Diener, D.R., and Rosenbaum, J.L. (2013). The cilium secretes bioactive ectosomes. Curr. Biol. *23*, 906–911.
6. Follit, J.A., Tuft, R.A., Fogarty, K.E., and Pazour, G.J. (2006). The intraflagellar transport protein IFT20 is associated with the Golgi complex and is required for cilia assembly. Mol. Biol. Cell *17*, 3781–3792.
7. Finetti, F., Paccani, S.R., Riparbelli, M.G., Giacomello, E., Perinetti, G., Pazour, G.J., Rosenbaum, J.L., and Baldari, C.T. (2009). Intraflagellar transport is required for polarized recycling of the TCR/CD3 complex to the immune synapse. Nat. Cell Biol. *11*, 1332–1339.
8. Nachury, M.V., Loktev, A.V., Zhang, Q., Westlake, C.J., Peranen, J., Merdes, A., Slusarski, D.C., Scheller, R.H., Bazan, J.F., Sheffield, V.C., *et al*. (2007). A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. Cell *129*, 1201–1213.
9. Jin, H., White, S.R., Shida, T., Schulz, S., Aguiar, M., Gygi, S.P., Bazan, J.F., and Nachury, M.V. (2010). The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. Cell *141*, 1208–1219.
10. Jones, R.F. (1970). Physiological and biochemical aspects of growth and gametogenesis in Chlamydomonas reinhardtii. Ann. NY Acad. Sci. *175*, 648–659.
11. Kubo, T., Kaida, S., Abe, J., Saito, T., Fukuzawa, H., and Matsuda, Y. (2009). The Chlamydomonas hatching enzyme, sporangin, is expressed in specific phases of the cell cycle and is localized to the flagella of daughter cells within the sporangial cell wall. Plant Cell Physiol. *50*, 572–583.
12. Young, R.W. (1971). The renewal of rod and cone outer segments in the rhesus monkey. J. Cell Biol. *49*, 303–318.
13. Young, R.W., and Bok, D. (1969). Participation of the retinal pigment epithelium in the rod outer segment renewal process. J. Cell Biol. *42*, 392–403.
14. Mullen, R.J., and LaVail, M.M. (1976). Inherited retinal dystrophy: primary defect in pigment epithelium determined with experimental rat chimeras. Science *192*, 799–801.
15. Chen, P., Hao, W., Rife, L., Wang, X.P., Shen, D., Chen, J., Ogden, T., Van Boemel, G.B., Wu, L., Yang, M., *et al*. (2001). A photic visual cycle of rhodopsin regeneration is dependent on Rgr. Nat. Genet. *28*, 256–260.
16. Allikmets, R., Shroyer, N.F., Singh, N., Seddon, J.M., Lewis, R.A., Bernstein, P.S., Peiffer, A., Zabriskie, N.A., Li, Y., Hutchinson, A., *et al*. (1997). Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science *277*, 1805–1807.
17. Hogan, M.C., Manganelli, L., Woolard, J.R., Masyuk, A.I., Masyk, T.V., Tammachote, R., Huang, B.Q., Leontrovich, A.A., Beito, T.G., Madden, B.J., *et al*. (2009). Characterization of PKD protein-positive exosomes-like vesicles. J. Am. Soc. Nephrol. *20*, 278–288.
18. Snell, W.J. (1976). Mating in Chlamydomonas: a system for the study of specific cell adhesion. I. Ultrastructural and electrophoretic analyses of flagellar surface components involved in adhesion. J. Cell Biol. *68*, 48–69.

Department of Biochemistry & Biophysics, UCSF Mission Bay, 600 16th St., San Francisco, CA 94143-2200, USA.
E-mail: Wallace.Marshall@ucsf.edu

http://dx.doi.org/10.1016/j.cub.2013.04.056

Coral Reefs: Building a Better Crystal Ball

Reef corals are ancient taxa, yet they are highly sensitive to environmental change. Recent research indicates that unless global CO_2 emissions are dramatically reduced, we are just decades away from the collapse of coral reef ecosystems.

John F. Bruno

> *"Predictive ecology threatens the ivory towers of academic ecology and may force ecologists to compete with engineers and other scientists on the unforgiving ground of real life rather than in the meadows of arcane theory." – R.H. Peters, "A Critique for Ecology" [1]*

Ecologists are understandably leery of prediction. In any given ecological system, there are millions of interacting parts that are constantly moving and evolving. There are nested feedbacks, multiple scales of interaction and unpredictable perturbations. Forecasting the result of tweaking such biological complexity is daunting. Yet, society needs ecologists to do exactly that: namely, to predict the ecological outcomes of policy decisions on everything from genetically modified crops to exotic species introductions. For example, we know that greenhouse gas emissions are having large and growing ecological impacts. But where are the tipping points? How much and how quickly do we need to reduce emissions to avoid catastrophic and irreversible ecological change? And how much, if at all, can local management increase system 'resilience' to climate change? A paper by Emma Kennedy, Peter Mumby and colleagues [2] in a recent issue of *Current Biology* tackles this challenge head on by modeling the fate of coral reefs under different emissions and management scenarios.

Coral reefs are being degraded by overfishing, disease and predator outbreaks, as well as various forms of pollution [3]. On top of this, CO_2 emissions are warming the ocean and also making it more acidic because the extra CO_2 reacts with seawater to form carbonic acid [4]. Scleractinian corals are the 'foundation species' [5] of tropical coral reefs. They secrete calcium carbonate skeletons that, over time, create vast structures that form the basis of complex ecosystems with thousands of species (Figure 1) [6]. Ocean warming is already killing corals [7] and laboratory experiments suggest that 'ocean acidification' will reduce the growth of coral colonies (and presumably whole-reef calcification) by roughly 25% by the end of this century. In combination, all of these stressors are thinning coral populations and reducing the structural complexity of the habitat corals create [8,9]. This has knock-on effects on fishes, invertebrates, and other reef inhabitants [10]. Additionally, reefs with little living coral quickly stop accreting vertically and begin to erode due to the actions of animals such as worms and sea urchins, called 'bioeroders', that burrow into and scrape away coral skeletons.

Disassembly Rules for Coral Reefs

Kennedy *et al.* [2] forecast the structural decay of Caribbean reefs based on emission scenarios from the new 'representative concentration